Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	493	carboxylesterase$1	US-PGPUB; USPAT	OR	OFF	2004/03/22 09:23
L2	9561	cpt-11 or (cpt adj "11") or apc	US-PGPUB; USPAT	OR	OFF	2004/03/22 09:23
L3	2210	camptothecin	US-PGPUB; USPAT	OR	OFF	2004/03/22 09:24
L4	147	1 same (2 or 3)	US-PGPUB; USPAT	OR	OFF	2004/03/22 09:25
L5	24	rabbit same 1	US-PGPUB; USPAT	OR	OFF	2004/03/22 09:25
L6	5	4 and 5	US-PGPUB; USPAT	OR	OFF	2004/03/22 09:26

PGPUB-DOCUMENT-NUMBER: 20030235811

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030235811 A1

TITLE: Crystallized mammalian carboxylesterase polypeptide and screening methods employing same

PUBLICATION-DATE: December 25, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 267756

DATE FILED: October 9, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60374513 20020422 US

US-CL-CURRENT: 435/4, 435/196 , 702/19

ABSTRACT:

Solved three-dimensional crystal structures of mammalian carboxylesterases (CEs) are disclosed. A solved three-dimensional crystal structure of a rabbit CE polypeptide co-crystallized with 4PP is disclosed. Solved three-dimensional structures of a human CE polypeptide co-crystallized with tacrine and a human CE polypeptide co-crystallized with homatropine are disclosed. The disclosed structures can be employed in the design of CE modulators. Methods of designing modulators of the biological activity of rabbit CE, human CE and other CE polypeptides, are also disclosed.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is based on and claims priority to U.S. Provisional Patent Application Serial No. 60/374,513, filed Apr. 22, 2002, the entire contents of which are herein incorporated by reference.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

Solved three-dimensional crystal structures of mammalian carboxylesterases (CEs) are disclosed. A solved three-dimensional crystal structure of a rabbit CE polypeptide co-crystallized with 4PP is disclosed. Solved three-dimensional structures of a human CE polypeptide co-crystallized with tacrine and a human CE polypeptide co-crystallized with homatropine are disclosed. The disclosed structures can be employed in the design of CE modulators. Methods of designing modulators of the biological activity of rabbit CE, human CE and other CE polypeptides, are also disclosed.

Summary of Invention - Table CWU - BSTL (1):
1 Abbreviations 4PP 4-piperidino-piperidine AcChE acetylcholinesterase ACE angiotension-converting enzyme AD Alzheimer's disease ADP adenosine diphosphate ATP adenosine triphosphate BSA bovine serum albumin .beta.ME .beta.-mercaptoethanol CE carboxylesterase CPT-11 irinotecan DMSO dimethyl sulfoxide DNA deoxyribonucleic acid DTT dithiothreitol EDTA ethylenediaminetetraacetic acid FAEE fatty acid ethyl esters hCE1 human carboxylesterase I hiCE human intestinal CE HEPES N-2-Hydroxyethylpiperazine-N'-2-ethanesulfoni- c acid kDa kilodalton(s) MAD multiwavelength anomalous diffraction MAN mannose NAG N-acetylglucosamine NDP nucleotide diphosphate nt nucleotide NTP nucleotide triphosphate PAGE polyacrylamide gel electrophoresis PCR polymerase chain reaction pI isoelectric point rhncCEH rat hepatic neutral cytosolic cholseteryl ester hydrolase rLCE rat lung carboxylesterase rLCE rat lung CE rhncCEH rat hepatic neutral cytosolic cholesteryl ester hydrolase RMSD root-mean-square deviation SDS sodium dodecyl sulfate SDS-PAGE sodium dodecyl sulfate polyacrylamide gel electrophoresis SIRAS single isomorphous replacement anomalous scattering SN-38 a potent topoisomerase I-specific poison SSRL Stanford Synchrotron Radiation Laboratory tAcChE Torpedo cailfornica acetylcholinesterase WT wildtype

Brief Description of Drawings Paragraph - DRTX (3):
[0029] FIG. 1 is a schematic depicting a general two-step activation of the anticancer topoisomerase I poison CPT-11 to SN-38 (an active metabolite) and 4-piperidino-piperidine (4PP) by carboxylesterases.

Brief Description of Drawings Paragraph - DRTX (6):
[0032] FIG. 3 is a ribbon diagram representing the structure of rabbit liver carboxylesterase indicating the three domains: a catalytic domain, an .alpha..beta. domain, and a regulatory domain. The catalytic domain is blue; the .alpha..beta. is green; and the regulatory domain is red. Catalytic residues are in green, N-linked glycosyl groups are in cyan and disulfide linkages are in orange.

Detail Description Paragraph - DETX (2):
[0044] SEQ ID NO: 1 is a DNA sequence encoding a rabbit carboxylesterase polypeptide.

Detail Description Paragraph - DETX (3):
[0045] SEQ ID NO: 2 is an amino acid sequence of a rabbit carboxylesterase polypeptide.

Detail Description Paragraph - DETX (16):
[0056] In one aspect, a mammalian carboxylesterase can cleave the anticancer prodrug CPT-11 (irinotecan), a potent topoisomerase I poison, into SN-38, an active metabolite, and 4-piperidino-piperidine (4PP). FIG. 1 depicts a generalized schematic of this process. 4-piperidino-piperidine-carboxylate spontaneously hydrolyzes to 4PP and CO.sub.2 after step 2, as depicted in FIG. 1.

Detail Description Paragraph - DETX (17):
[0057] The 2.5 .ANG. crystal structure of rabbit liver carboxylesterase (rCE) is the most efficient enzyme known to activate CPT-11 in this manner, in complex with the leaving group 4PP. 4PP is observed bound adjacent to a high-mannose Asn-linked glycosylation site on the surface of rCE. This product-binding site is separated from the catalytic gorge by a thin wall of amino acid side chains, suggesting that 4PP could be released through this

secondary product exit pore. In accordance with the present invention, the crystallographic observation of a leaving group bound on the surface of rCE supports the "back door" product exit site proposed for the acetylcholinesterases. Thus, the present invention facilitates the design of improved anticancer drugs or enzymes for use in viral-directed cancer cotherapies.

Detail Description Paragraph - DETX (21):
[0061] In one embodiment, the crystal structure of the carboxylesterase from rabbit liver was determined to 2.54 .ANG. resolution. This structure was determined by crystallizing purified rabbit carboxylesterase, obtaining x-ray diffraction data from these crystals and solving the crystal structure by employing the combined methods of molecular replacement and crystallographic refinement/model building.

Detail Description Paragraph - DETX (22):
[0062] The crystal structure of rabbit liver carboxylesterase is of interest for several reasons, including, but not limited to the following. First, this enzyme activates that anticancer drug CPT-11 to SN-38, a potent topoisomerase I poison. Rabbit liver carboxylesterase is the most efficient enzyme known in this activation process. Understanding how rabbit carboxylesterase activates CPT-11 will help elucidate how human enzymes perform this process, which can lead to improved anticancer drugs. Next, rabbit liver carboxylesterase is highly similar in sequence (81% identity at the amino acid level) to the human carboxylesterase 1 that metabolizes cocaine, heroin, many drugs, xenobiotics and organophophorus compounds, and is involved in cholesterol and fatty acid metabolism and hormone production in humans. Further, carboxylesterases are required to activate the cholesterol-lowering statin drugs (e.g., lovastatin). Thus, rabbit liver carboxylesterase can be used to model how these processes occur in humans and to develop improved drugs.

Detail Description Paragraph - DETX (23):
[0063] Prior to the present disclosure, the molecular basis for activation of CPT-11 to SN-38 was unknown. In addition, the detailed molecular processes for the breakdown of narcotics, cholesterol and certain hormones by carboxylesterases, were also unknown. This is the first crystal structure of a mammalian carboxylesterase. This structure can be used to analyze how drugs (particularly, but not limited to, CPT-11, cocaine and heroin), cholesterol, fatty acid, hormones, and other xenobiotic compounds are processed in humans.

Detail Description Paragraph - DETX (24):
[0064] Additionally, a crystalline structure of the present invention can be used to generate more effective CPT-11 anticancer drugs. Knowledge of how mammals activate CPT-11 to SN-38 by cleaving a carboxylester linkage can facilitate the design of improved CPT-11 analogues that are more easily activated. In addition, the crystal structure of rabbit liver carboxylesterase can facilitate the design of inhibitors to related enzymes (like human butylcholinesterases) that would be useful in reducing the side effects of CPT-11 treatments. Further, this invention can be used to generate inhibitors of carboxylesterases useful in treating narcotic and alcohol overdoses. Inhibitors of carboxylesterases can be given to overdose victims to reduce the metabolism of cocaine and heroin, thus reducing the production of dangerous metabolites.

Detail Description Paragraph - DETX (56):
[0095] As used herein, the term "CE" is used to refer to a carboxylesterase (CE) polypeptide that can bind CPT-11 and/or one or more ligands, and to nucleic acids encoding the same. The term "CE" includes invertebrate homologs; however, CE nucleic acids and polypeptides can also be isolated from vertebrate

sources. "CE" further includes vertebrate homologs of CE family members, including, but not limited to, mammalian and avian homologs. Representative mammalian homologs of CE family members include, but are not limited to, rabbit, murine and human homologs.

Detail Description Paragraph - DETX (76):

[0114] Expression of rCE in human tumor cell lines and in xenografts grown in immune-deprived mice sensitizes them to CPT-11. Potter et al., (1998) Cancer Res. 58: 2646-2651; Danks et al., (1999) Clin. Cancer Res. 5: 917-924; Danks et al., (1998) Cancer Res. 58: 20-22; Potter et al., (1998) Cancer Res. 58: 3627-3632. Viral-based gene therapy approaches have also demonstrated promise for providing an efficient, targeted way to activate CPT-11 in humans. Weirdl et al., (2001) Cancer Res. 61: 5078-5082; Meck et al., (2001) Cancer Res. 61: 5083-5089. For example, adenoviruses expressing rCE can sensitize tumor cells to CPT-11 up to 127-fold, and a secreted form of the protein can elicit a bystander effect to cells not expressing the enzyme. Weirdl et al., (2001) Cancer Res. 61: 5078-5082. Additionally, ex vivo purging approaches to eliminate neuroblastoma cells from bone marrow have been designed and are now being tested for clinical utility. Meck et al., (2001) Cancer Res. 61: 5083-5089. Ultimately, rCE could prove useful in sensitizing human tumors to CPT-11 or other ester-linked prodrugs. Thus, an aspect of the present invention is to provide the first structural view of a mammalian carboxylesterase and insights into CPT-11 activation.

Detail Description Paragraph - DETX (289):

[0315] Thus, the first crystallographic evidence of product bound adjacent to a putative esterase secondary exit channel is presented herein. These results advance understanding of esterase function and the ability of mammalian carboxylesterases to act on a wide variety of substrates. In addition, these results facilitate the design of novel CPT-11 analogs or engineered forms of rCE for use in cancer chemotherapy.

Detail Description Paragraph - DETX (295):

[0319] Diffraction data were collected at Stanford Synchrotron Radiation Laboratory (SSRL) beamline 9-1, and were processed and reduced using MOSFILM (Leslie, (1992), Joint CCP4+ESF-EAMCB Newsletter on Protein Crystallography, No. 26.). Crystals were of space group P2.sub.1, and contained six molecules in the asymmetric unit for both the tacrine and homatropine complexes. See FIG. 12, in which each hCE1 is depicted in a different color. The structures of hCE1 were determined by molecular replacement using the structure of rabbit carboxylesterase (rCE; RCSB Protein ID No. 1K4Y; Bencharit et al., (2002) Nat.Struct.Biol. 9: 337), also an aspect of the present invention, as a search model (81% sequence identity). Non-identical side chains were trimmed prior to rotation and translation function searches in AmoRe (Navaza & Saludjian, (1997) Methods Enzymol. 276A: 581-594). The structures were refined using torsion angle dynamics in CNS with the maximum likelihood function target, and included an overall anisotropic B-factor and a bulk solvent correction. Non-crystallographic symmetry restraints were used at early stages of refinement, and then removed such that six independent molecules were refined for both the tacrine and homatropine complexes. 10% of the observed data were set aside for cross-validation using free-R prior to refinement. Manual adjustments and rebuilding were performed using the program O (Jones et al., (1991) Acta Crystallogr. A 47: 110-119) and .sigma..sub.A-weighted electron density maps (Read, (1986) Acta Crystallogr. A 42: 140-149). At the later stages of refinement the N-linked glycans and waters were added. Tacrine was placed in multiple orientations at the active site of hCE1 using standard and simulated annealing difference maps, as well as computational results from BLOB. Homatropine was placed at the active site of hCE1 using standard and simulated annealing difference maps, and at the surface site using similar maps

and guidance from BLOB computational results. The final hCE1 structures (FIGS. 11-14) were evaluated by PROCHECK (Laskowski et al., (1993) J. Appl. Crystallogr. 26: 283-291), and exhibit good geometry. As depicted in FIG. 11, hCE1 appears to adopt a trimeric configuration. In FIG. 11, each hCE is depicted in green, red and blue. The an region is depicted in green, the regulatory domain is depicted in red and the catalytic domain is depicted in blue.

Detail Description Table CWU - DETL (2):

5TABLE 2 Crystallographic Data And Refinement For Rabbit Carboxylesterase In Complex With 4PP Resolution.sup.1 (.ANG.; highest shell) 20-2.5 (2.54-2.5) Space Group R32 Cell Constants (.ANG.) a = b = 110.23; c = 282.52 Total Reflections 234,266 Unique Reflections 22,041 Mean Redundancy 10.6 Wilson B-factor (.ANG..sup.2) 41.1 R.sub.sym (%).sup.1,2 7.2 (42.1) Completeness.sup.1 (%) 99.7 (99.1) Mean I/.sigma..sup.1 31.7 (4.5) R.sub.cryst (%).sup.3 22.8 R.sub.free (%).sup.4 29.2 RMSD.sup..sctn. Bond Lengths (.ANG.) 0.0067 RMSD.sup..sctn. Bond Angles (.degree.) 1.34 RMSD.sup..sctn. Dihedrals (.degree.) 22.9 RMSD.sup..sctn. Impropers (.degree.) 0.91 Number of Atoms.sup.5 Protein 3,897 (60.9) Solvent 388 (57.5) Carbohydrate 99 (89.9) Ligand 24 (75.9) .sup.1The number in parentheses is for the highest resolution shell. .sup.2R.sub.sym = .SIGMA. .vertline.I - <I >.vertline./.SIGMA.I- , where I is the observed intensity and <I> is the average intensity of several symmetry-related observation of that reflection. .sup.3R.sub.cryst = .SIGMA. .parallel.F.sub.o.vertline. .vertline. - .vertline.F.sub.c.parallel./.SIGMA. .vertline.F.sub.o.vertline., where F.sub.o and F.sub.c are the observed and calculated structure factors, respectively. .sup.4R.sub.free = .SIGMA. .parallel.F.sub.o.vertl- ine. - .vertline.F.sub.c.parallel./.SIGMA. .vertline.F.sub.o.vertline. for 10% of the data not used at any stage of structural refinement. .sup.5The number in parentheses is the mean B-factor (.ANG..sup.2). .sup..sctn.RMSD, root mean square deviation.

PGPUB-DOCUMENT-NUMBER: 20030198595

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030198595 A1

TITLE: Use of bi-specific antibodies for pre-targeting diagnosis and therapy

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 150654

DATE FILED: May 17, 2002

RELATED-US-APPL-DATA:

child 10150654 A1 20020517

parent continuation-in-part-of 09382186 19990823 US PENDING

child 10150654 A1 20020517

parent continuation-in-part-of 09823746 20010403 US PENDING

non-provisional-of-provisional 60104156 19981014 US

non-provisional-of-provisional 60090142 19980622 US

US-CL-CURRENT: 424/1.49, 530/391.1 , 534/11

ABSTRACT:

The present invention relates to a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable construct. The targetable construct comprises a carrier portion which comprises or bears at least one epitope recognizable by at least one arm of said bi-specific antibody or antibody fragment. The targetable construct further comprises one or more therapeutic or diagnostic agents or enzymes. The invention provides constructs and methods for producing the bi-specific antibodies or antibody fragments, as well as methods for using them.

[0001] This application is a continuation-in-part of U.S. Ser. No. 09/382,186, filed Aug. 23, 1999 and a continuation-in-part of U.S. Ser. No. 09/823,746, filed Apr. 3, 2001, both of which are continuations-in-part of U.S. Ser. No. 09/337,756, filed Jun. 22, 1999, the contents of which are incorporated herein by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (53):
[0213] The prodrug CPT-11 (irinotecan) is converted in vivo by carboxylesterase to the active metabolite SN-38. One application of the invention, therefore, is to use a bsAb targeted against a tumor and a hapten (e.g. di-DTPA) followed by injection of a di-DTPA-carboxylesterase conjugate. Once a suitable tumor-to-background localization ratio has been achieved, the CPT-11 is given and the tumor-localized carboxylesterase serves to convert CPT-11 to SN-38 at the tumor. Due to its poor solubility, the active SN-38 will remain in the vicinity of the tumor and, consequently, will exert an effect on adjacent tumor cells that are negative for the antigen being targeted. This is a further advantage of the method. Modified forms of carboxylesterases have been described and are within the scope of the invention. See, e.g., Potter et al., Cancer Res. 58:2646-2651 (1998) and Potter et al., Cancer Res. 58:3627-3632 (1998).

Detail Description Paragraph - DETX (54):
[0214] Etoposide is a widely used cancer drug that is detoxified to a major extent by formation of its glucuronide and is within the scope of the invention. See, e.g., Hande et al. Cancer Res. 48:1829-1834 (1988). Glucuronide conjugates can be prepared from cytotoxic drugs and can be injected as therapeutics for tumors pre-targeted with mAb-glucuronidase conjugates. See, e.g., Wang et al. Cancer Res. 52:4484-4491 (1992). Accordingly, such conjugates also can be used with the pre-targeting approach described here. Similarly, designed prodrugs based on derivatives of daunomycin and doxorubicin have been described for use with carboxylesterases and glucuronidases. See, e.g., Bakina et al. J. Med Chem. 40:4013-4018 (1997). Other examples of prodrug/enzyme pairs that can be used within the present invention include, but are not limited to, glucuronide prodrugs of hydroxy derivatives of phenol mustards and beta-glucuronidase; phenol mustards or CPT-11 and carboxypeptidase; methotrexate-substituted alpha-amino acids and carboxypeptidase A; penicillin or cephalosporin conjugates of drugs such as 6-mercaptopurine and doxorubicin and beta-lactamase; etoposide phosphate and alkaline phosphatase.

Detail Description Paragraph - DETX (248):
[0349] Two vials of rabbit liver carboxylesterase (SIGMA; protein content.about.17 mg) are reconstituted in 2.2 ml of 0.1 M sodium phosphate buffer, pH 7.7 and mixed with a 25-fold molar excess of CA-DTPA using a freshly prepared stock solution (.about.25 mg/ml) of the latter in DMSO. The final concentration of DMSO in the conjugation mixture is 3% (v/v). After 1 hour ofrincubation, the mixture is pre-purified on two 5-mL spin-columns (Sephadex G50/80 in 0.1 M sodium phosphate pH 7.3) to remove excess reagent and DMSO. The eluate is purified on a TSK 3000G Supelco column using 0.2 M sodium phosphate pH 6.8 at 4 ml/min. The fraction containing conjugate is concentrated on a Centricon-10.TM. concentrator, and buffer-exchanged with 0.1 M sodium acetate pH 6.5. Recovery: 0.9 ml, 4.11 mg/ml (3.7 mg). Analytical HPLC analysis using standard conditions, with in-line UV detection, revealed a major peak with a retention time of 9.3 min and aminor peak at 10.8 min in 95-to-5 ratio. Enzymatic analysis showed 115 enzyme units/mg protein, comparable to unmodified carboxylesterase. Mass spectral analyses (MALDI mode) of both unmodified and DTPA-modified CE shows an average DTPA substitution ratio near 1.5. A metal-binding assay using a known excess of indium spiked with radioactive indium confirmed the DTPA:enzyme ratio to be 1.24 and 1.41 in duplicate experiments. Carboxylesterase-DTPA is labeled with In-111 acetate at a specific activity of 12.0 mCi/mg, then treated with excess of non-radioactive

indium acetate, and finally treated with 10 mM EDTA to scavenge off excess non-radioactive indium. Incorporation by HPLC and ITLC analyses is 97.7%. A HPLC sample is completely complexed with a 20-fold molar excess of bi-specific antibody hMN-14 Fab'.times.734 Fab', and the resultant product further complexes with WI2 (anti-ID to hMN-14), with the latter in 80-fold molar excess with respect to bi-specific antibody.

PGPUB-DOCUMENT-NUMBER: 20030031681

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030031681 A1

TITLE: Combined growth factor-deleted and thymidine kinase-deleted vaccinia virus vector

PUBLICATION-DATE: February 13, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 991721

DATE FILED: November 13, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60137126 19990528 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
US	PCT/USOO/14679	2000US-PCT/USOO/14679	May 26, 2000

US-CL-CURRENT: 424/186.1, 435/235.1 , 435/456

ABSTRACT:

A composition of matter comprising a vaccinia virus expression vector with a negative thymidine kinase phenotype and a negative vaccinia virus growth factor phenotype.

RELATED APPLICATIONS

[0001] This application claims the benefit of priority from PCT/US00/14679, filed May 26, 2000, which claims the benefit of priority from U.S. Provisional Patent Application No. 60/137,126, filed May 28, 1999, each of which is hereby incorporated by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (48):

[0064] Several other suicide gene systems have been recently described. Thymidine phosphorylase, which catalyzes the reversible phosphorolytic cleavage of thymidine, deoxyuridine and their analogs, has been used to convert the prodrug 5'-deoxy-5-fluorouridine to 5-FU. Cytosine arabinoside (ara-C) requires phosphorylation by deoxycytidine kinase (dCK) to form its active metabolite. Delivery of dCK to glioma cells sensitized them to treatment by ara-C. Overexpression of a rabbit carboxylesterase was shown to sensitize human cells to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (CPT-11) by its conversion to an active metabolite (SN38). Both .beta.-glucosidase and the plant equivalent linamarase have been shown to

hydrolyse amygdalin and linamarase respectively to cyanide. This leads to tumor specific toxicity when delivered via antibody-targeting or retroviral transduction, with no noted systemic toxicity.

Detail Description Table CWU - DETL (2):

2TABLE 2 Enzyme/Prodrug Systems ENZYME PRODRUG ACTIVE DRUG Herpes Simplex Virus Gancyclovir Gancyclovir triphosphate thymidine kinase Varicella Zoster Virus (E)-5-(2-bromovinyl)-2'- BVDU triphosphate thymidine kinase deoxyuridine (BVDU) Cytosine deaminase 5-fluorocytosine 5-fluorouracil Purine nucleoside 6-methylpurine deoxyriboside 6-methylpurine phosphorylase .beta.-lactamase 7-(4-carboxybutanamido)- phenylenediamine mustard cephalosporin mustard Carboxypeptidase G2 4-[(2-chloroethyl)(2- 4-[(2-chloroethyl)(2- mesyloxyethyl)amino] mesyloxyethyl)amino] benzoic benzoyl-L-glutamic acid acid (CJS11) (CMDA) Cytochrome P450-2B1 Cyclophosphamide/ifosfamide acrolein + phosphoramide mustard E. coli nitroreductase CB1954 (S-aziridin--yl-2-4- 5-aziridin-1-yl-4- dinitrobenzamide) hydroxylamino-2- nitrobenzamide Xanthine-guanine 6-thioxanthine 6-thioxanthine monophosphate phosphoribosyl-transferase .beta.-glucuronidase epirubicin-glucoronide Epirubicin Thymidine phosphorylase 5'-deoxy-5-fluorouridine 5-fluorouracil Deoxycytidine kinase Cytosine arabinoside Cytosine ababinoside monophosphate Carboxylesterase 7-ethyl-10-[4-(1-piperidino)-1- 7-theyl-10- piperidino] hydroxycamptothecin (SN-38) carbonyloxycamptothecin (CPT-11) Linamarase/.beta.-glucosidase Linamarin/Amygdalin Cyanide Carboxypeptidase A Methotrexate-phenylalamine Methotrexate Cytochrome P450-4B1 2-aminoanthracene, 4- unknown alkylating agents ipomeanol

US-PAT-NO: 6602499

DOCUMENT-IDENTIFIER: US 6602499 B1

TITLE: Combination viral-based and gene-based therapy of tumors

DATE-ISSUED: August 5, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 302952

DATE FILED: April 30, 1999

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/083,663, filed on Apr. 30, 1998, which is herein incorporated by reference.

US-CL-CURRENT: 424/93.2, 424/93.1 , 435/320.1 , 435/325 , 514/44

ABSTRACT:

The present invention relates to viral mutants and methods of using these viral mutants for selectively killing neoplastic cells. The viral mutants of the invention are capable of selectively killing neoplastic cells by a combination of viral mediated oncolysis and anti-cancer ("suicide") gene therapy.

30 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Detailed Description Text - DETX (32):

Chemotherapeutic agents for use in the invention should not significantly inhibit replication of the viral mutant so as to allow the viral mutant to kill tumor cells by viral oncolysis, as well as by delivery of the suicide gene. The use of a chemotherapeutic agent/transgene combination in which the chemotherapeutic agent, or its active metabolites, act instead by crosslinking DNA or by inhibiting DNA repair would not significantly inhibit replication of the viral mutant. Thus, such chemotherapeutic agent/transgene combinations are encompassed by the viral mutant and methods of the present invention. A preferred chemotherapeutic agent/transgene combination is cytochrome P450 combined with CPA, ifosfamide, N-methyl cyclophosphamide, MCPNU, or polymeric forms of: CPA, ifosfamide, N-methyl cyclophosphamide and MCPNU. A more preferred chemotherapeutic agent/transgene combination is CPA/cytochrome P450 2B 1. Other chemotherapeutic agent/transgene combinations for use in the

present invention include: CB1954/E. coli nitroreductase (Friedlos et al., Gene Ther. 5: 105-112 (1998); Green et aL, Cancer Gene Ther. 4: 229-238 (1997)); topoisomerase I or II inhibitors/enzyme with esterase-like activity, such as, e.g., CPT-11/carboxylesterase (Jansen et al., Int. J. Cancer 70. 335-340 (1997); Danks et al., Cancer Res. 58. 20-22 (1998)); 4-ipomeanol/cytochrome P450 4B1 (Verschoyle et al., Toxicol. Appl. Pharmacol. 123: 193-198 (1993)); and 2-aminoanthracene/cytochrome P450 4B1 (Smith et al., Biochem. Pharmacol. 50: 1567-1575 (1995)).

Detailed Description Text - DETX (151):

It was also important to determine the effect of the converted CPA on viral replication. In previous experiments, the combination of hrR3 and ganciclovir provided a significant anticancer effect in an 9L tumor model. (Boviatsis et al., Cancer Res. 54: 5745-5751 (1994)). However, the converted ganciclovir molecules also inhibit viral replication, therefore, use of ganciclovir/thymidine kinase may not be a good selection in this paradigm. The results in the examples show that CPA/CYP2B1, while providing an anticancer effect, does not significantly inhibit viral protein synthesis or viral replication. The explanation for this finding may lie in the mode of action of CPA's active metabolite, phosphoramide mustard (PM). PM produces interstrand and intrastrand crosslinks in the genome of cells: maximum cytotoxicity to cellular DNA is usually achieved during mitosis when multiple DNA strand breaks occur at the cross-link sites (Colvin, in Cancer Medicine, Holland et al., eds., Lea and Fabiger, Phila., pub., 1993, at 733-734). Instead, non-mitotic, cross-linked viral DNA may be spared from extensive damage and may be thus be repaired more readily than genomic DNA. Other chemotherapeutic agent/transgene combinations in which the active metabolites would not be expected to significantly inhibit replication of the viral vector include: topoisomerase I or II inhibitors/enzyme with esterase-like activity, such as, e.g., CPT-11/carboxylesterase (Jansen et al., Int. J Cancer 70. 335-340 (1997); Danks et al., Cancer Res. 58: 20-22 (1998)); CB1954/E. coli nitroreductase (Friedlos et al., Gene Ther. 5: 105-112 (1998); Green et al., Cancer Gene Ther. 4: 229-238 (1997)); 4-ipomeanol/cytochrome P450 4B1 (Verschoyle et al., Toxicol. Appl. Pharmacol. 123: 193-198 (1993)); and 2-aminoanthracene/cytochrome P450 4B 1 (Smith et al., Biochem. Pharmacol. 50: 1567-1575 (1995)). These chemotherapeutic agent/gene combinations act by inhibiting DNA repair or by DNA-alkylation, respectively.

Other Reference Publication - OREF (48):

Danks, M.K., et al., "Overexpression of a Rabbit Liver Carboxylesterase Sensitizes Human Tumor Cells in CPT-11," Cancer Res. 58:20-22 (Jan. 1998).

US-PAT-NO: 6361774

DOCUMENT-IDENTIFIER: US 6361774 B1

TITLE: Methods and compositions for increasing the target-specific toxicity of a chemotherapy drug

DATE-ISSUED: March 26, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 399221

DATE FILED: September 17, 1999

US-CL-CURRENT: 424/178.1, 424/179.1 , 424/181.1 , 424/9.1 , 530/391.1

ABSTRACT:

A method for increasing the target-specific toxicity of a drug is effected by pretargeting an enzyme to a mammalian target site, and then administering a cytotoxic drug known to act at the target site or a prodrug form thereof which is converted to the drug in situ, which drug is also detoxified to form an intermediate of lower toxicity using said mammal's ordinary metabolic processes, whereby the detoxified intermediate is reconverted to its more toxic form by the pretargeted enzyme and thus has enhanced cytotoxicity at the target site. Further enhancement can be achieved by pretargeting an enzyme which converts the prodrug to the cytotoxic drug at the target site. Kits for use with the method also are provided. The method and kits permit lower doses of cytotoxic agents, maximize target site activity and minimize systemic side effects.

7 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (46):

The prodrug CPT-11 (irinotecan) is converted in vivo by carboxylesterase to the active metabolite SN-38. One application of the invention, therefore, is to use a bsMAb targeted against a tumor and a hapten (e.g. DTPA) followed by injection of a DTPA-carboxyl esterase conjugate. Once a suitable tumor-to-background localization ratio has been achieved, the CPT11 is given and the tumor-localized carboxylesterase serves to convert CPT-11 to SN-38 at the tumor. Since the active SN-38 is poorly soluble it will remain in the vicinity of the tumor and, since it is being generated in the vicinity of the tumor, it is able to exert an effect on adjacent tumor cells that are negative for the antigen being targeted. These are further advantages of the method. Modified forms of carboxylesterase that can be expressed by cells have been described (Potter et al., Cancer Res., 58:2646-2651 and 3627-3632, 1998), and such designed enzymes are within the scope of the invention.

Brief Summary Text - BSTX (47):

Etoposide is a widely used cancer drug that is detoxified to a major extent by formation of its glucuronide (Hande et al., Cancer Res., 48: 1829-1834, 1988), and could therefore be used within the scope of the invention. Glucuronide conjugates can be prepared from cytotoxic drugs and be injected as therapeutics for tumors pre-targeted with MAb-glucuronidase conjugates (Wang et al., Cancer Res., 52:4484-4491, 1992). Accordingly, such conjugates can also be used with the bsMAb approach described here. Designed prodrugs based on derivatives of daunomycin and doxorubicin have been described (Bakina et al., J. Med Chem., 40:4013-4018, 1997) for use with carboxylesterases and glucuronidases, and these can be used within the scope of the invention. Some other combinations of prodrugs and enzymes that can be used within the invention are listed. Glucuronide prodrugs of hydroxy derivatives of phenol mustards (Schmidt et al., Bioorg. Med Chem. Lett., 7:1071-1076, 1997) and beta-glucuronidase. Phenol mustards or CPT-11 and carboxypeptidase. Methotrexate-substituted alpha-amino acids and carboxypeptidase A. Beta-lactamase and penicillin or cephalosporin conjugates of drugs such as 6-mercaptopurine and doxorubicin. Alkaline phosphatase and etoposide phosphate.

Brief Summary Text - BSTX (49):

The clearance characteristics of drugs can be modulated by certain agents, and the use of such modulating agents within the invention form an additional embodiment. For example, CPT-11 clearance properties have been shown to be modulated by administration of cyclosporin A with the latter reducing the level of biliary clearance of SN-38 and its glucuronide (SN-38G) (Gupta et al., Cancer Res. 56:1309-1314, 1996). In turn, this raised the plasma concentration of SN-38G. This would allow for greater contact with tumor-targeted DTPAglucuronidase in the present invention. Gupta et al. also showed a similar effect when using phenobarbitol (Cancer Chemother. Pharmacol., 39:440-444, 1997), and thus, this agent could also be given along with CPT-11 after pre-targeting DTPA-glucuronidase. In the latter article they also showed that pretreatment of rats with valproic acid (an inhibitor of uridine diphosphate glucuronosyl transferase (UDP-GT) inhibited the formation of SN-38G leading to a 270% AUC for SN-38 from subsequently-administered CPT-11. Thus, use of valproic acid, within the scope of the invention when pre-targeting DTPA-carboxylesterase to tumor, will also lead to higher levels of SN-38 at the target.

Other Reference Publication - OREF (4):

Potter, et al. "Isolation and Partial Characterization of a cDNA Encoding a Rabbit Liver Carboxylesterase That Activates the Prodrug Irinotecan (CPT-11)" Cancer Res., 58:2646-2651, 1998.

Other Reference Publication - OREF (5):

Potter, et al. "Cellular Localization Domains of a Rabbit and Human Carboxylesterase: Influence on Irinotecan (CPT-11) Metabolism by the Rabbit Enzyme" Cancer Res., 58:3627-3632, 1998.

Other Reference Publication - OREF (13):

Hiromitsu Takayama et al., "Synthesis of a New Class of Camptothecin Derivatives, The Long-Chain Fatty Acid Esters of 10-Hydroxycampothecin, As A Potent Prodrug Candidate, and Their In Vitro Metabolic Conversion By Carboxylesterases", Bioorganic & Medicinal Chemistry Letters, Oxford, Great Britain, vol. 8, No. 5, (Mar. 3, 1998), (415-418).

Other Reference Publication - OREF (14):

XP-000867715 Mary K. Danks et al., "Comparison of Activation of CPT-11 By Rabbit and Human Carboxylesterases for Use in Enzyme/Prodrug Therapy", Clinical

Cancer Research, (Apr. 1999), 5(4), (917-924).